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SCIENTIFIC DATA REVIEWS
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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
CHEMICAL SAFETY AND
POLLUTION PREVENTION

MEMORANDUM

Date: July 28, 2011

SUBJECT: An Oral (Gavage) Acute Neurotoxicity Comparison in Rodents

PC Codes: 129013, 097805, 004006, 109709 109701, 128722, 004005
Decision No.: 444059
Petition No.: NA
Risk Assessment Type: NA
TXR No.: 0055614

MRID No.: 48333801

DP Barcode: D385562

Registration No.: NA
Regulatory Action: NA
Case No.: NA
CAS Nos.: 39515-40-7, 52918-63-5, 72963-72-5, 240494-70-6, 52645-53-1, 23031-36-9, 584-79-2
40 CFR: NA

FROM: Marquea King, Ph.D., Toxicologist
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THROUGH: Christine Olinger, Acting Branch Chief
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I. CONCLUSIONS

The study evaluated the acute neurotoxic potential of the test substances in an enhanced functional observational battery (FOB) using similar methodology and dosing regimens for each test substance.

FOB Pyrethroid Classification		
Test article	**Structural element**	**FOB Classification**
Vehicle	Corn oil	-
Metofluthrin	Non-cyano	Type I
Imiprothrin	Non-cyano	Type I
Permethrin [a]	Non-cyano	Type I
Prallethrin	Non-cyano	Type I
Cyphenothrin	Cyano	Mixed
Deltamethrin [a]	Cyano	Type II
Pynamin Forte (d-allethrin)	Non-cyano	Type I

a= Permethrin and Deltamethrin represented Type I and Type II positive control groups, respectively.

II. ACTION REQUESTED

Review Sumitomo submission of a report titled: "Classification of Seven Pyrethroids based on FOB study in rats" (MRID 48363201) to follow up with their earlier submission beaned on 1/7/11, under D385562, MRID: 48333801.

III. BACKGROUND

The acute neurotoxicity comparison study in rodents was submitted to support the hypothesis that the pyrethroid class of insecticides exerts their toxicological effect through at least two distinct mechanisms of action operating at the level of sodium and calcium ion channels (N, P/Q- and T types). In the study, which evaluated enhanced FOB parameters specific to pyrethroid chemicals, 5 pyrethroids were evaluated. The FOB parameters in the study were modified to include additional details (e.g., the coarseness of tremor) to distinguish findings particularly associated with pyrethroid intoxication. The pyrethroids tested in the study included: cyphenothrin, imiprothrin, metofluthrin, prallethrin, and pynamin forte (deltamethrin and permethrin were included as positive controls). A similar study (MRID 47050505) is also available that evaluates 12 additional pyrethroids. Both studies were conducted in the same manner using the same laboratory, identical laboratory procedures and FOB screening criteria Therefore, the results between the two studies are directly comparable.

Pyrethroid	PC Code	CAS No.
Cyphenothrin	129013	39515-40-7
Deltamethrin	097805	52918-63-5
Imiprothrin	004006	72963-72-5
Metofluthrin	109709	240494-70-6
Permethrin	109701	52645-53-1
Prallethrin	128722	23031-36-9
Pynamin Forte	004005	584-79-2

IV. RESULTS

FOB findings noted in this study consisted of known effects of pyrethroid compounds. All compounds elicited CNS and neuromuscular effects (tremors, clonic convulsions, low arousal, impaired mobility and/or gait and posture abnormalities) to different degrees. Slight or moderately coarse tremors were generally observed in all test substances, however more severe tremors were observed in Metofluthrin and Prallethrin treated rats. Pynamin Forte treated rats were most affected by clonic convulsions. The most frequent altered gait observed was splayed or dragging hind limbs (Metofluthrin, Cyphenothrin and Deltamethrin) and ataxia (Metofluthrin, Imiprothrin, Permethrin, Prallethrin and Deltamethrin). Postural abnormalities and impaired mobility were observed in response to treatment with all test substances except Imiprothrin.

Physiological effects (red deposits on or around the nose and mouth and differences in mean body temperature), sensorimotor effects (no response to approach, touch, tail pinch and pupil stimuli; a more energetic response to startle stimulus; and/or a lack of olfactory orientation) were observed in the Metofluthrin, Cyphenothrin, Permethrin and Deltamethrin groups. Effects on autonomic endpoints (evidence of decreased grooming, lacrimation and/or chromodacryorrhea) were observed in Imiprothrin and Deltamethrin groups.

DATA EVALUATION RECORD

Pyrethroid Chemicals:
Cyphenothrin, Imiprothrin, Metofluthrin, Prallethrin, Pynamin Forte, Permethrin, Deltamethrin

PC Code: 129013,004006,109709,128722,004005,109701, 097805
TXR#: 0055614
MRID#: 48333801

An Oral (Gavage) Acute Neurotoxicity Comparison Study in Rats

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Contract Number: EP-W-10013
Work Assignment No.: WA-0-01

Task No.: 0-1-32
EPA WAM/Reviewer: Myron Ottley / Edward Scollon

This review may be altered by EPA subsequent to the contractors' signatures above.

EPA Reviewer: Marquea D. King, Ph.D. **Signature:**
Registration Action Branch IV, Health Effects Division (7509P) Date: 8/1/11
EPA Work Assignment Manager: Lori Brunsman **Signature:**
Science Information Management Branch, Health Effects Division (7509P) Date: 8/9/11

TXR: 0055614

DATA EVALUATION RECORD

STUDY TYPE: non-guideline (Acute Neurotoxicity) - Rats 870.6200a ['81-8]; OECD 424

PC CODE: 129013, 004005 004006, 109709, 128722, 109701, 097805

DP BARCODE: 386418

TEST MATERIAL: Cyphenothrin, Deltamethrin, Imiprothrin, Metofluthrin, Permethrin, Prallethrin, and Pynamin Forte (d-allethrin) (all purities not identified)

CITATION: Herberth, M.T. (2010) An Oral (Gavage) Acute Neurotoxicity Comparison Study in Rats. WIL Research Laboratories, LLC, 1407 George Road, Ashland, OH 44805-9281. Laboratory report number: WIL-118041, December 20, 2010. MRID 48333801.Unpublished.

SPONSOR: Sumitomo Chemical Company. Ltd. 27-1, Shinkawa 2-chom, Chuo-ku, Tokyo 104-8260, Japan

EXECUTIVE SUMMARY:

The objective of the study was to evaluate the acute neurotoxic potential of the test substances in a functional observational battery (FOB) comparison study using similar methodology and dosing regimens for each test substance.

In an acute neurotoxicity study (MRID 483338-01) (Non-guideline), groups of 10 male Crl:CD(SD) rats received a single dose of one of seven (7) pyrethroid compounds by oral gavage followed by testing in a Functional Observational Battery (FOB) at the time of peak effect for each compound. Dose levels and times of peak effect were selected based on the results of a previous range-finding acute FOB comparison study (Knapp, 2009, W1L-118040). One to three doses were evaluated for each test substance. Each group of 10 male rats received a single dose of a single test compound (Cyphenothrin (60, 100 or 160 mg/kg), Imiprothrin (900, 1200 or 1500 mg/kg), Metofluthrin (57 or 75 mg/kg), Prallethrin (150, 250 or 400 mg/kg) or Pynamin Forte (d-allethrin) (200, 320 or 500 mg/kg). Two additional test substances (Permethrin (200 mg/kg) and Deltamethrin (25 mg/kg)) were Type I and Type II positive controls, respectively. Control groups received corn oil (vehicle). The dose volume was 5 mL/kg.

Animals were observed twice daily for mortality and moribundity. All animals found dead or *in extremis* were subjected to gross necropsy. Clinical examinations were performed on all animals prior to euthanasia. FOB findings were recorded for all animals at the time of peak effect (2 to 4 hr) on study day 0. Surviving animals were euthanized within 24 hr of completion of FOB and were discarded without macroscopic examination.

FOB findings noted in this study consisted of known effects of pyrethroid compounds. All compounds elicited CNS and neuromuscular effects (tremors, clonic convulsions, low arousal,

impaired mobility and/or gait and posture abnormalities) to different degrees. Slight or moderately coarse tremors were generally observed in all test substances, however more severe tremors were observed in Metofluthrin and Prallethrin treated rats. Pynamin Forte/d-allethrin treated rats were most affected by clonic convulsions. The most frequent altered gait observed was splayed or dragging hind limbs (Metofluthrin, Cyphenothrin and Deltamethrin) and ataxia (Metofluthrin, Imiprothrin, Permethrin, Prallethrin and Deltamethrin). Postural abnormalities and impaired mobility were observed in response to treatment with all test substances except Imiprothrin.

Physiological effects (red deposits on or around the nose and mouth and differences in mean body temperature), sensorimotor effects (no response to approach, touch, tail pinch and pupil stimuli; a more energetic response to startle stimulus; and/or a lack of olfactory orientation) were observed in the Metofluthrin, Cyphenothrin, Permethrin and Deltamethrin groups. Effects on autonomic endpoints (evidence of decreased grooming, lacrimation and/or chromodacryorrhea) were observed in Imiprothrin and Deltamethrin groups.

Specific effects associated with each test substance are summarized as follows:

***Metofluthrin* (57 and 75 mg/kg doses):** Two animals in the high dose (75 mg/kg) Metofluthrin group were found dead; all others survived to scheduled euthanasia. Severe tremors were noted within 3 hours of dosing for 2 additional rats in the high dose group. One of these two males also had an unkempt appearance and red material around his nose. No clinical findings were noted in the low dose (57 mg/kg) group rats. Home cage parameter effects and slight tremors were observed in both dose groups. Abnormal handling observations included increased respiratory rate in both dose groups. Dose-dependent effects on open field parameters in the 57 and 75 mg/kg groups included moderately to totally impaired mobility; slight to markedly coarse tremors, considerably to severely impaired gait, and low arousal. 75 mg/kg males also showed dragging body, splayed or dragging hind limbs and/or were ataxic. The number of rats without tumors was significantly lower than controls in the 75 mg/kg group. Also mean rearing counts and mean time to first step were significantly different from controls in the 57 and 75 mg/kg groups. Test substance-related effects on sensory parameters were noted in the 75 mg/kg dose group; and more energetic responses to the startle stimulus were observed in both the 57 and 75 mg/kg groups. Abnormal neuromuscular observations in the 57 and 75 dose groups included reduced or absent hind limb resistance. Mean rotarod performance in the 75 mg/kg groups was lower than controls. A test substance related increase in mean catalepsy time was observed in rats in the 75 mg/kg dose group compared to the control group.

***Imiprothrin* (900, 1200 and 1500 mg/kg doses):** All rats in all three Imiprothrin dose groups survived to scheduled euthanasia. Clinical findings occurring prior to the 2 hr FOB assessment consisted of moderate to severe tremors in all three dose groups. Severe salivation was noted in one male in the high group that had tremors. No other clinical findings were noted at any dose. Test substance related changes in home cage parameters were observed in rats in all three dose groups. Abnormal handling observations included decreased grooming. Open field observations noted one rat in the 1500 mg/kg group had a hunched body and 1 rat in the 1200 mg/kg group exhibited slightly impaired mobility, ataxia, moderately coarse tremors and considerable gait impairment. Clonic convulsions were noted for one rat in the 900 mg/kg group. Sensory parameters were not affected in any of the three Imiprothrin dose groups. Mean hind limb grip strength for the 1500 mg/kg groups was lower than the control group. No test substance related physiological effects were noted.

Permethrin (200 mg/kg dose): All rats in the single, 200 mg/kg Permethrin group survived to scheduled euthanasia. No clinical findings were noted. Abnormal handling observations included increased respiratory rates and red deposits around the nose. Test substance-related findings from the open field observations included slightly impaired mobility, ataxia and/or slight to moderately coarse tremors. Significantly increased mean time to first step, grooming counts and lower mean rearing counts were also noted. Altered sensory parameters noted in rats in the Permethrin dose group included no response during touch, no pupil response and lightly uncoordinated air righting reflex. Mean forelimb grip strength in the 200 mg/kg groups was significantly lower than the control group. A slight increase in mean body temperature was noted in Permethrin dosed rats.

***Prallethrin* (150, 250 and 400 mg/kg):** All rats in the three Prallethrin dose groups survived to scheduled euthanasia. One rat in the high dose group had an unkempt appearance. No other clinical findings were observed. Home cage parameters, including clonic convulsions, flattened posture, splayed hind legs and/or slight to coarse tremors, were observed in the mid-level 250 mg/kg dose group. A single rat in the high (400 mg/kg) dose group and one in the low 150 mg/kg dose group showed altered home cage behaviors. Abnormal handling observations included red deposits around nose in the 250 and 400 mg/kg groups. Slightly to moderately impaired mobility, clonic convulsions, slight to moderately coarse tremors, ataxia and/or slightly to considerably impaired gait were noted in the 250 and 400 mg/kg groups. No test substance-related altered sensory observations, neuromuscular parameters or physiological effects were observed in any of the three Prallethrin dose groups.

Cyphenothrin (60, 100 and 160 mg/kg doses): Two rats in the high (160 mg/kg) Cyphenothrin dose group with multiple signs of toxicity were found dead on study day 1. Two additional rats in the high group had severe tremors; one was euthanized *in extremis*. All other rats in all dose groups survived to scheduled euthanasia and showed no clinical signs related to test substance administration. Significantly more rats in the 160 mg/kg dose group had slight to moderately coarse tremors compared to controls. Clonic convulsions and splayed hind limbs were also observed. A single rat in each of the 60 and 100 mg/kg dose groups also showed altered home cage behavior. Abnormal handling observations included increased respiratory rates in the 100 and 160 mg/kg groups and tense and hard muscle tone was observed in the 160 mg/kg group. Significantly higher numbers of rats in the 160 mg/kg group had slightly impaired mobility and slightly or moderately coarse tremors. Slightly to considerably impaired gait was also increased in the 160 mg/kg group. Splayed or dragging hind limbs, clonic convulsions, impaired gait, slightly impaired mobility and moderately coarse tremors were also noted in the 100 mg/kg groups. A dose related decrease in mean rearing counts was noted in the 100 and 160 mg/kg dose groups and a significant increase in urination was observed in the 160 mg/kg group. Abnormal sensory observations in the 160 mg/kg dose group included a more energetic response to startle stimulus and one rat had no hind limb extension. Mean rotarod performance was lower in the 160 mg/kg dose group compared to the control group. Dose-related increases in mean catalepsy time were noted for rats in the 100 and 160 mg/kg dose groups compared to controls. No other test substance-related physiological parameters were observed.

Deltamethrin (25 mg/kg dose): All rats in the single, 25 mg/kg Deltamethrin dose group survived to scheduled euthanasia. Clonic convulsions were observed in one rat, and two other rats had unkempt appearances. No other test substance related clinical findings were noted. A significant increase in the number of males with postural abnormalities and slight tremors were also observed. Abnormal handling observations included significant decreases in normal fur appearance and decreased grooming; along with significant increases in chromodacryorrhea, red deposits around the nose and abdominogenital wetness. Significant differences from the control

group were observed for moderately impaired mobility and ataxia. Mean rearing count was also lower in the treated animals. The number of rats with abnormal air-righting reflex was significantly higher in the dosed group compared to the control group. Two rats also had no hind limb extension and exhibited no response following approach stimulation; while one rat had a more energetic response to a startle stimulus. Mean forelimb and hind limb grip strength was lower, and mean catalepsy time was increased compared to controls in the 25 mg/kg dose group. A significantly lower mean body temperature was also observed in the treated group.

Pynamin Forte/d-allethrin (200, 320 and 500 mg/kg doses): Three rats in the high (500 mg/kg) Pynamin Forte dose group and one rat in the medium (320 mg/kg) dose group were found dead on study day 1. All other rats survived to scheduled euthanasia. Moderate tremors were observed in one rat in each of the two highest dose groups. No other test substance related clinical findings were observed. A dose response increase in slight or moderately coarse tremors was observed in rats in all three of the dose groups. The number of slight tremors observed in rats in the 500 mg/kg dose group was statistically significantly higher than in control rats. Flattened posture splayed hind legs and clonic convulsions were also observed in the 320 and 500 mg/kg dose groups. Abnormal handling observations included significant increases in the number of males with increased respiratory rate, red deposits around nose and tense and hard muscle tone in the high dose group. The most prevalent test substance-related open field findings in all three dose groups included slightly impaired mobility, clonic convulsions, slight to moderate tremors and slight gait impairment. These findings were significantly increased compared to controls. Also, dose related, significant increases in mean time to first step and significant decreases in mean rearing counts were observed in the 320 and 500 mg/kg groups. No test-substance related effects were observed on sensory parameters in any of the Pynamin Forte dose groups. Rats in all three Pynamin Forte dose groups, however, showed reduced hind limb resistance, as well as decreased forelimb and hind limb grip strength compared to controls. Non-statistically significant, but test substance-related increases in mean catalepsy time was noted in rats in the 320 and 500 mg/kg dose groups compared to controls.

This neurotoxicity study is classified as **Acceptable/Non-guideline** and does not satisfy the guideline requirement for an acute neurotoxicity study in rats (870.6200; OECD 424). The study fulfilled the purpose for which it was intended, evaluation of the acute neurotoxic potential of the test articles in a FOB comparison using the same methodology and dosing regimen.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

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I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Cyphenothrin

Description:	Dark yellow, clear viscous liquid
Lot/batch #:	081001
Purity	95.1%

Test material: Imiprothrin

Description:	Clear, brown viscous liquid
Lot/batch #:	081003
Purity	94.0%

Test material: Metofluthrin

Description:	Pale, clear yellow liquid
Lot/batch #:	95.6%

Test material: Prallethrin

Description:	Dark brown viscous liquid
Lot/batch #:	080607
Purity	93.6%

Test material: Pynamin Forte/d-allethrin

Description:	Dark yellow, clear liquid
Lot/batch #:	080505

Test material: Permethrin

Description:	Light brown, clear viscous liquid
Lot/batch #:	PL07-0347
Purity	95.1%

Test material: Deltamethrin

Description:	Off-white powder
Lot/batch #:	27500029
Purity	99.2%

2. Vehicle: Corn oil, NF (Spectrum Chemical Manufacturing Corporation, New Brunswick, NJ).

3. Test animals:

Species:	Rat (males only)
Strain:	Crl: CD®(SD)
Age/weight at dosing:	6 weeks; 147-240 g
Source:	Charles River Laboratories, Inc., Raleigh, NC
Housing:	Individually in wire-mesh cages suspended above cage-board
Diet:	Certified Rodent LabDiet® 5002, PMI Nutrition International, Inc. *ad libitum*
Water:	Reverse-osmosis treated municipal water, *ad libitum*
Environmental conditions:	**Temperature:** 22±3 °C **Humidity:** 50±20 % **Air changes:** At least 10/hr **Photoperiod:** 12 hrs dark/ 12 hrs light
Acclimation period:	Minimum of 13 days

B. STUDY DESIGN:

1. **In life dates:** Start: March 26, 2010; End: December 20, 2010.

2. **Animal assignment and treatment:** Animals were assigned to the test groups noted in Table 1 by a computerized random sort program based on body weight such that individual body weight was within ±20% of the mean. Rats were given a single dose by gavage in corn oil at a volume of 5 mL/kg followed by FOB testing at the time of peak effect. Dose levels and time to peak effect were chosen based on the results of a previous study (Knapp, 2009, WIL-118040) and were provided by the Sponsor Representative after consultation with the WIL Study Director. Following the FOB, survivors were sacrificed and discarded without necropsy. The study was conducted in blocks of one to three test articles; each block had a concurrent control group. Each control and treated group consisted of 10 males. Animals were not fasted prior to treatment to avoid any potential confounding effects of inanition on the behavior of the test subjects.

TABLE 1. Study Design for Male Rats Treated with a Pyrethroid

Test article	Dose Levels (mg/kg)	Dosage Concentration (mg/ml)	Number of Males	Time of Peak Effect (hr)
Vehicle	0	0	10/time	2,4
Metofluthrin	57, 75	11.4, 15	10/dose	2
Imiprothrin	900, 1200, 1500	180, 240, 300	10/dose	2
Permethrin [a]	200	40	10/dose	4
Prallethrin	150, 250, 400	30, 50, 80	10/dose	2
Cyphenothrin	60, 100, 160	12, 20, 32	10/dose	2
Deltamethrin [a]	25	5	10/dose	4
Pynamin Forte/d-allethrin	200, 320, 500	40, 64, 100	10/dose	2

a= Permethrin and Deltamethrin represented Type I and Type II positive control groups, respectively.

3. **Test substance preparation and analysis**: Dose formulations were prepared once as single formulations for each dose level on the day prior to or the day of dosing, divided into aliquots for dispensation, and stored refrigerated (approximately 4 °C), protected from light. The test substance formulations were stirred continuously throughout the preparation, sampling, and dose administration procedures. Homogeneity, stability, or concentration of the test substance formulated in the vehicle were assessed prior to initiation of dose administration and were verified by the Sponsor. Homogeneity and stability assessments were not performed on the positive control groups, Deltamethrin and Permethrin.

Results

Homogeneity and concentration analysis: The analyzed dosing formulations were met the WIL standard procedure (SOP) requirement and were within the acceptable range for suspensions (85% to 115% of target concentrations). No test substance was detected in the analyzed vehicle formulations.

Stability analysis: The analyzed dosing formulations were stable for 8 hours at room temperature and 8 days when refrigerated.

The analytical data indicated that the mixing procedure was adequate and the test compound was stable and homogeneous under conditions of this study.

4. **Statistics:** Data are presented as mean ± SD (standard deviation) and the number of animals used to calculate the mean. All statistical tests were performed using WTDMS™ unless otherwise noted. Analyses were conducted using two-tailed tests (except as noted otherwise) for minimum significance levels of 1% and 5%, comparing each test substance- and positive control-treated group to the respective control group.

   Post-dosing continuous FOB data for the test substance-treated groups were subjected to a parametric one-way ANOVA (Snedecor and Cochran, 1980) to determine intergroup differences. If the ANOVA revealed significant ($p<0.05$) intergroup variance, Dunnett's test (Dunnett, 1964) was used to compare the test substance-treated groups to the respective control group. For the Permethrin and Deltamethrin positive control-treated groups, data for the aforementioned parameters were compared to the respective control group using a two-sample t-test (Snedecor and Cochran, 1980). FOB parameters that yielded scalar or descriptive data were analyzed using Fisher's Exact Test (Steel and Torrie, 1980).

C. METHODS / OBSERVATIONS:

1. **Clinical Observations and Survival:** Animals were observed twice daily for mortality and morbidity. Detailed clinical examinations were performed on all animals prior to euthanasia.

2. **Body Weight:** Individual body weights were recorded on study day 0 prior to test substance administration. These body weights were recorded for the purpose of dose calculations only.

3. **Food Consumption:** Food consumption was not measured.

4. **Functional Observational Battery (FOB) Assessments:** FOB findings were recorded for all animals at the time of peak effect on study day 0. The time of peak effect was considered to be approximately 2 and 4 hours post-dosing for test and positive control substances, respectively, based on FOB findings of increased respiration, impaired mobility, altered gait (body dragging, splayed or dragging hind limbs, ambulating on tiptoes, and ataxia), convulsions, tremors, and/or low arousal observed at the appropriate time point in a previous range-finding acute FOB comparison study (Knapp, 2009, WIL-118040). The FOB used at WIL Research is based on previously developed protocols (Gad, 1982; Haggerty, 1989; Irwin, 1968; Moser et al, 1988; Moser et al., 1991, and O'Donoghue, 1989). Testing was performed by the same technicians, to the extent possible, without knowledge of the animal's group assignment. All animals were observed for the following parameters as described in Table 2 below:

TABLE 2. Standard FOB Parameters					
X	HOME CAGE OBSERVATIONS	X	HANDLING OBSERVATIONS	X	OPEN FIELD OBSERVATIONS
X	Posture	X	Ease of removal from cage	X	Mobility
X	Biting	X	Ease of handling in hand	X	Rearing
X	Convulsions	X	Lacrimation/ chromodacryorrhea	X	Arousal/ general activity level
X	Tremors	X	Salivation	X	Convulsions
X	Palpebral (eyelid) closure	X	Piloerection	X	Tremors
X	Feces consistency	X	Fur appearance	X	Abnormal movements
		X	Palpebral closure	X	Urination / defecation
		X	Respiratory rate	X	Grooming
	SENSORY OBSERVATIONS	X	Red/crusty deposits	X	Gait abnormalities / posture
X	Approach response	X	Mucous membranes /eye /skin color	X	Gait score
X	Touch response	X	Eye prominence	X	Bizarre / stereotypic behavior
X	Startle response	X	Muscle tone	X	Backing
X	Pupil response		PHYSIOLOGICAL OBSERVATIONS	X	Time to first step (seconds)
X	Eyeblink response	X	Body weight		
X	Forelimb extension	X	Body temperature		NEUROMUSCULAR OBSERVATIONS
X	Hindlimb extension	X	Catalepsy	X	Hindlimb extensor strength
X	Air righting reflex		OTHER OBSERVATIONS	X	Forelimb grip strength
X	Olfactory orientation			X	Hindlimb grip strength
X	Tail pinch response			X	Hindlimb foot splay
				X	Rotarod performance

5. **Macroscopic Examination (Unscheduled Death):** A complete necropsy was conducted on all animals found dead or euthanized *in extremis*, moribund animals were euthanized by carbon dioxide inhalation. The necropsy included examination of the external surface, all orifices, and the cranial, thoracic, abdominal, and pelvic cavities, including viscera. No tissue samples were preserved.

6. **Study Termination**: All surviving animals were euthanized by carbon dioxide inhalation and discarded without macroscopic examination within 24 hours of the final FOB assessments.

II. RESULTS:

A. MORTALITY: A total of six rats were found dead during the study and one was euthanized *in extremis*. Clinical findings were summarized in Table 3.

Metofluthrin: Two males (nos. 72321 and 72348) in the 75 mg/kg Metofluthrin group were found dead 2 and 3 hours following dose administration. These deaths were considered to be test substance-related as these males had multiple signs of overt toxicity during the 2-hour FOB evaluation. All other males in the 75 mg/kg Metofluthrin group survived to the scheduled euthanasia. Severe tremors were noted within 3 hours of dose administration for an additional 2 males in the75 mg/kg Metofluthrin group (Table 3). One of these males also had an unkempt appearance and red material around the nose the following morning.

All males in the 57 mg/kg Metofluthrin group survived to the scheduled euthanasia; no clinical findings were noted.

Imiprothrin: All males in the 900, 1200, and 1500 mg/kg Imiprothrin groups survived to the scheduled euthanasia. Clinical findings occurring prior to the 2-hour FOB assessment consisted of moderate to severe tremors for 1, 3, and 3 males in the 900, 1200, and 1500 mg/kg groups, respectively, shortly after dose administration (within 1 hour 15 minutes) (Table 3). In addition, severe salivation was noted on the day of dose administration for a male in the 1500 mg/kg group that had tremors (Table 3). No other clinical findings were noted at any dose level.

Permethrin: All males in the 200 mg/kg Permethrin group were survived to the scheduled euthanasia. No clinical findings were noted.

Prallethrin: All males in the 150, 250, and 400 mg/kg Prallethrin groups survived to the scheduled euthanasia. Clinical findings of moderate tremors and body twitches were noted prior to the 2-hour FOB assessment (within 1 hour 40 minutes of dose administration) for 1 male each in the 250 and 400 mg/kg groups. Another male in the 400 mg/kg Prallethrin group had an unkempt appearance the following morning. No other test substance-related clinical findings were observed at any dose level.

Cyphenothrin: Two males (nos. 72329 and 72430) in the 160 mg/kg Cyphenothrin group were found dead on study day 1 (Table 3). These deaths were considered to be test substance-related as these males had multiple signs of overt toxicity during the 2-hour FOB evaluation. Male no. 72366 in 160 mg/kg group was noted with severe tremors at 3 hours 24 minutes following dose administration and was subsequently euthanized in extremis (Table 3). All other males in the 160 mg/kg Cyphenothrin group survived to the scheduled euthanasia; the only clinical findings were severe tremors and a twitching body noted in male no. 72380 in the 160 mg/kg Cyphenothrin group approximately 3 hours 30 minutes following dose administration. All males in the 60 and 100 mg/kg Cyphenothrin groups survived to the scheduled euthanasia; the only clinical finding noted (hair loss right forelimb) was considered to be a common finding in laboratory animals.

Deltamethrin: All males in the 25 mg/kg Deltamethrin group survived to the scheduled euthanasia. Clonic convulsions were noted for male no. 72377 in this group prior to the 4-hour FOB assessment (at 3 hours 2 minutes following dose administration). In addition, male nos. 72377 and 72423 had unkempt appearances on study day 1. The only other clinical

finding noted (hair loss on the forelimbs) were considered to be a common finding in laboratory animals.

Pynamin Forte/d-allethrin: In the Pynamin Forte groups, male nos. 72769 (320 mg/kg) and 72753, 72820, and 72824 (500 mg/kg) were found dead between 2 hours and 3 hours following dose administration, or on study day 1. Male no. 72769 in the 320 mg/kg group was prostrate and had moderate tremors following the 2-hour FOB assessment (approximately 3 hours following dosing) and was found dead shortly thereafter. With the exception of male no. 72820 that was found dead during the FOB assessment, and had limited FOB findings, the Pynamin Forte-treated males that were found dead had overt signs of toxicity during the 2-hour FOB evaluation. All other males in the 320 and 500 mg/kg Pynamin Forte groups survived to the scheduled euthanasia. Moderate tremors were noted for 1 male (no. 72823) in the 500 mg/kg Pynamin Forte group at 1 hour 20 minutes following dose administration (Table 3). The only other clinical finding noted (hair loss on the forelimbs) in the 320 mg/kg/day group was considered to be a common finding in laboratory animals. All males in the 200 mg/kg Pynamin Forte group survived to the scheduled euthanasia; no clinical findings were noted for any of these males.

TABLE 3. Summary of Clinical Findings

Test Substance	Dose Level (mg/kg)	Clinical Parameter (Total occurrence/No. of animals)						
		Number Found Dead	Number Euthanized *in Extremis*	Tremor	Unkempt Appearance	Dried Red Nose	Salivation	Body Twitches
Metofluthrin	57	0	0	0/0	0/0	0/0	0/0	0/0
	75	2	0	2/2	1/1	1/1	0/0	0/0
Imiprothrin	900	0	0	1/1	0/0	0/0	0/0	0/0
	1200	0	0	3/3	0/0	0/0	0/0	0/0
	1500	0	0	3/3	0/0	0/0	1/1	0/0
Permethrin	200	0	0	0/0	0/0	0/0	0/0	0/0
Prallethrin	150	0	0	0/0	0/0	0/0	0/0	0/0
	250	0	0	1/1	0/0	0/0	0/0	0/0
	400	0	0	1/1	1/1	0/0	0/0	1/1
Cyphenothrin	60	0	0	0/0	0/0	0/0	0/0	0/0
	100	0	0	0/0	0/0	0/0	0/0	0/0
	160	2	1	2/2	0/0	0/0	0/0	1/1
Deltamethrin	25	0	0	0/0	2/2	0/0	0/0	0/0
Pynamin Forte	200	0	0	0/0	0/0	0/0	0/0	0/0
	320	1	0	1/1	0/0	0/0	0/0	0/0
	500	3	0	1/1	0/0	0/0	0/0	0/0

Data were obtained from Tables S1, S2 and S3 on page 83-85 of the study report.

B. BODY WEIGHT:

Mean body weights for all test substance-related groups were similar to the respective control group prior to dose administration (Table 4).

TABLE 4. Summary of Body Weight (g±SD)

Test Substance	Dose Level (mg/kg)	Body Weight (g) (n=10)
Vehicle	0	188±22.5
Metofluthrin	57	185±18.4
	75	187±19.3

Imiprothrin	900	188±23.9
	1200	185±21.9
	1500	190±21.9
Permethrin	200	188±22.8
Vehicle	0	208±22.9
Prallethrin	150	205±16.6
	250	208±18.5
	400	208±21.0
Cyphenothrin	60	208±19.9
	100	208±21.8
	160	201±23.3
Deltamethrin	25	206±22.5
Vehicle	0	196±13.6
Pynamin Forte	200	195±17.6
	320	195±17.2
	500	197±15.1

Data were obtained from Tables S4, S5 and S6 on page 86-88 of the study report.
None were significantly different from control.

C. FOB FINDINGS:

1. Home Cage Observations (see Table 5):

Metofluthrin: Test substance-related effects on home cage parameters were noted for the 2 males found dead in the 75 mg/kg Metofluthrin group. Flattened posture (limbs may have been extended); splayed hind legs, extremely or moderately coarse tremors, and/or biting of the cage were noted for these males approximately 2 hours following dose administration. In addition, slight tremors were noted for 3 and 2 surviving males in the 57 and 75 mg/kg Metofluthrin groups, respectively. No other remarkable findings were noted in the 75 mg/kg Metofluthrin group.

Imiprothrin: One male in 900 mg/kg Imiprothrin group was noted with rearing and a twitching head and/or body approximately 2 hours following dose administration. In the 1200 mg/kg group, 1 male had flattened posture (limbs may have been extended), moderately coarse tremors, and was observed biting the cage. Slight tremors and/or biting of the cage were noted for 2 males in the 1500 mg/kg Imiprothrin group. These findings were considered to be test substance-related.

Permethrin: Slight or moderately coarse tremors were noted for 2 males in the 200 mg/kg Permethrin group. No other test substance-related findings were noted in the Permethrin-treated group during the home cage observations.

Prallethrin: Clonic convulsions (repetitive movement of the mouth and jaws), flattened posture (limbs may have been extended); splayed hind legs, and/or slight tremors were noted for 2 males in the 250 mg/kg Prallethrin group. In addition, 1 male in this group was noted sitting with the head held low and had markedly coarse tremors; a single male in the 400 mg/kg group was also noted sitting with the head held low. A single occurrence of slight tremors was noted for 1 male in the 150 mg/kg Prallethrin group.

Cyphenothrin: Slight or moderately coarse tremors were noted for 5 males in the 160 mg/kg Cyphenothrin group, resulting in significantly ($p<0.05$) fewer males without tremors in this group compared to the control group. Clonic convulsions, consisting of both repetitive

movement of the mouth and jaws and a twitching head and/or body, and splayed hind limbs were also noted for 1 of the males with moderately coarse tremors; this male was found dead following the 2-hour FOB evaluation. Another male in the 160 mg/kg Cyphenothrin group was noted sitting with the head held low and was observed biting the cage; this animal was ultimately euthanized in extremis. In the 60 and 100 mg/kg Cyphenothrin groups, a single male in each group was observed sitting with its head held low or had flattened posture; the 2 males also had clonic convulsions (head and/or body twitches) and/or slight tremors.

No other test substance-related findings were noted in the Cyphenothrin-treated groups during the home cage observations. An increase (significant, $p<0.05$) in the number of males with eyelids wide open was noted in the 60 mg/kg Cyphenothrin group compared to the control group; however, because wide-open eyes are generally considered to be normal, the increased incidence was not considered to be test substance-related.

Deltamethrin: Significant ($p<0.05$) increases in the number of males with postural abnormalities were noted in the 25 mg/kg Deltamethrin group compared to the control group. Five males in this group were noted with flattened posture (limbs may have been extended) and splayed hind legs. Two of these males were also noted with slight tremors. No other test substance-related findings were noted in the Deltamethrin-treated group at the home cage observations.

Pynamin Forte/d-allethrin: Slight or moderately coarse tremors were noted for 2, 4, and 8 males in the 200, 320, and 500 mg/kg Pynamin Forte groups, respectively; differences from the control group were significant ($p<0.05$) for slight tremors in the 500 mg/kg Pynamin Forte group. As a result, significantly ($p<0.05$) fewer males without tremors were noted in the 500 mg/kg Pynamin Forte group compared to the control group. In conjunction with the aforementioned tremors, flattened posture (limbs may have been extended) was noted for 1 male in each of the Pynamin Forte-treated groups (including 2 of the 4 males found dead), and splayed hind legs or clonic convulsions (head and/or body twitches) were noted for 2 males in the 500 mg/kg group. Clonic convulsions (back muscle twitches) were also observed in 1 male in the 320 mg/kg Pynamin Forte group. No other test substance-related findings were noted in the Pynamin Forte-treated group at the home cage observations.

TABLE 5. . Summary Findings of Home Cage Observations (no. animals showing sign)							
Treatment group	**Flattened Posture**	**Splayed Hind legs**	**Tremors**	**Rearing**	**Biting**	**Convulsions**	**Sitting, Head held low**
Number Tested	10	10	10	10	10	10	10
Metofluthrin, 57 mg/kg	0	0	3	0	0	0	0
Metofluthrin , 75 mg/kg	2	1	2	0	1	0	0
Imiprothrin, 900	0	0	0	1	0	1	0
Imiprothrin, 1200 mg/kg	1	0	1	0	1	0	0
Imiprothrin, 1500 mg/kg	0	0	2	0	1	0	1
Permethrin, 200 mg/kg	0	0	2	0	0	0	0
Prallethrin, 150 mg/kg	0	0	1	0	0	0	0
Prallethrin, 250 mg/kg	1	1	3	0	0	1	1
Prallethrin, 400 mg/kg	0	0	0	0	0	0	1
Cyphenothrin, 60 mg/kg	0	0	1	0	0	1	1
Cyphenothrin, 100 mg/kg	1	0	1	0	0	0	0
Cyphenothrin, 160 mg/kg	0	1	5	0	1	2	1
Deltamethrin, 25 mg/kg	5*	5*	2	0	0	0	2
Pynamin Forte, 200 mg/kg	1	0	2	0	0	0	0
Pynamin Forte, 320 mg/kg	1	0	3	0	0	1	0
Pynamin Forte, 500 mg/kg	1	1	6*	0	0	1	0

* Significantly different from control group at 0.05 using Fisher's Exact test.
Data were obtained from Tables S7, S8 and S9 on page 89-94 of the study report.

2. <u>Handling Observations (see Table 6)</u>:

<u>Metofluthrin</u>: One and 3 males in the 57 and 75 mg/kg Metofluthrin groups, respectively, had increased respiratory rate. Red deposits on or around the nose were also noted for 1 of the 75 mg/kg males with an increased respiratory rate. There were no other notable findings for the Metofluthrin-treated groups during the handling observations.

<u>Imiprothrin</u>: Evidence of decreased grooming, including slightly soiled fur, ventral wetness, and/or abdominogenital wetness, was noted for 2-3 males each in the 900, 1200, and 1500mg/kg Imiprothrin groups. These findings were considered to be test substance-related. In addition, 1 male in each of these groups was noted with red deposits on or around the nose and/or mouth. No other test substance-related effects on handling parameters were noted for males treated with Imiprothrin. In the 900 mg/kg Imiprothrin group, 1 male had an increased respiratory rate; however, because similar findings were generally not noted at higher dose levels, no relationship to treatment was apparent. In addition, removal of animals from their cages was easy (without vocalization, and with slight or no resistance) or difficult (runs around the cage or was hard to grab) for approximately half of the males in the 900, 1200, and 1500 mg/kg Imiprothrin groups; however, because these findings were noted at a similar incidence in the control group, they were not considered to be test substance-related.

<u>Permethrin:</u> Increased respiratory rate was noted for 3 males in the Permethrin-treated group during the handling observations. In addition to increased respiratory rates, red deposits on or around the nose were noted for 2 of these males. These findings were

consistent with the expected effect of the positive control substance. No other test substance-related effects on handling parameters were noted for males treated with Permethrin.

Prallethrin: One and 2 males in the 250 and 400 mg/kg Prallethrin groups, respectively, were noted with red deposits on or around the nose at the handling observations. No other test substance-related effects on handling parameters were noted for males treated with Prallethrin. One male in the 250 mg/kg group had an increased respiratory rate. However, because this finding was noted at a similar incidence in the control group and/or did not occur in a dose-related manner, it was not considered to be test substance-related.

Cyphenothrin: One and 2 males in the 100 and 160 mg/kg Cyphenothrin groups, respectively, had increased respiratory rate; one of the males in the 160 mg/kg group was subsequently euthanized in extremis. An additional 2 males in the 160 mg/kg group had tense and hard muscle tone. No other test substance-related effects on handling parameters were noted for males treated with Cyphenothrin.

Deltamethrin: In the 25 mg/kg Deltamethrin group, the number of males with ventral wetness was significantly ($p<0.05$) increased compared to the concurrent control group, resulting in a corresponding reduction (significant at $p<0.05$) in the number of males with normal fur appearance. Abdominogenital wetness was noted for an additional male in the 25 mg/kg Deltamethrin group. In addition to decreased grooming, chromodacryorrhea, slight lacrimation, red deposits on or around the nose, and/or freezing upon attempting to remove from cage were observed for 1-2 animals each. No other test substance-related effects on handling parameters were noted for males treated with Deltamethrin.

Pynamin Forte: A significantly ($p<0.05$) higher number of males in the 500 mg/kg Pynamin Forte group had an increased respiratory rate compared to the control group. As a result, a corresponding significant ($p<0.05$) reduction in the number of males with a normal respiratory rate was noted in this group. In addition, 3 males in the 500 mg/kg Pynamin Forte group had red deposits on or around the nose and 2, 1, and 3 males in the 200, 320, and 500 mg/kg Pynamin Forte groups, respectively, had tense and hard muscle tone. No other test substance-related effects on handling parameters were noted for males treated with Pynamin Forte.

TABLE 6 . Summary Findings of Handling Observations (no. animals showing sign)

Treatment group	Fur appearance	Increased respirator y rate	Red deposits (nose)	Muscle tone	Chromod-acryorrhea	Lacrimation	Ease removal (freezes)
Number Tested	10	10	10	10	10	10	10
Metofluthrin, 57 mg/kg	0	1	0		0	0	0
Metofluthrin , 75 mg/kg	0	3	1	0	0	0	0
Imiprothrin, 900 mg/kg	3	0	1	0	0	0	0
Imiprothrin, 1200 mg/kg	2	0	2	0	0	0	0
Imiprothrin, 1500 mg/kg	2	0	2	0	0	0	0
Permethrin, 200 mg/kg	0	3	2	1	0	0	0
Prallethrin, 150 mg/kg	0	0	0	0	0	0	0
Prallethrin, 250 mg/kg	0	1	1	0	0	0	0
Prallethrin, 400 mg/kg	0	2	2	2	0	0	0
Cyphenothrin, 60 mg/kg	0	0	0	0	0	0	0
Cyphenothrin, 100 mg/kg	0	1	0	0	0	0	0
Cyphenothrin, 160 mg/kg	0	2	0	2	0	0	0
Deltamethrin, 25 mg/kg	6	0	1	0	2	1	1
Pynamin Forte, 200 mg/kg	0	0	0	2	0	0	0
Pynamin Forte, 320 mg/kg	0	0	0	1	0	0	0

Pynamin Forte, 500 mg/kg	0	4	3	3	0	0	0

Data were obtained from Tables S10, S11 and S12 on page 95-103 of the study report.

3. Open Field Observations (see Table 7):

Metofluthrin: Test substance-related effects on open field parameters were noted in a dose-dependent manner for the 57 and 75 mg/kg groups. Moderately to totally impaired mobility, slight to markedly coarse tremors, considerably to severely impaired gait (gait score), and/or low arousal (somewhat stuporous) were noted for 2 and 5 males in the respective groups. The 75 mg/kg group males with impaired gait also had a dragging body, splayed or dragging hind limbs, and/or were ataxic; 2 of these males were found dead following the FOB evaluation. Due to the number of animals in the 75 mg/kg group that were noted with tremors (5 males), the number without tremors (5 males) in this group was significantly ($p<0.05$) lower when compared with the control group (10 males). In addition to the aforementioned findings, the mean time to first step was increased in a dose-related manner for the 57 and 75 mg/kg groups. Although the differences from the control group were not statistically significant, they were considered test substance-related. Moreover, mean rearing counts for the 57 and 75 mg/kg groups were lower than the control group; the differences were significant ($p<0.05$).

Imiprothrin: One male in the 1500 mg/kg Imiprothrin group was noted with a hunched body during the open field observations, while 1 male in the 1200 mg/kg group exhibited slightly impaired mobility, ataxia, moderately coarse tremors, and considerable gait impairment (without falling). Although these findings did not occur in a dose-related manner, based on the severity of the effects and the correlation to other test substance-related findings, the open field observations noted in the 1200 mg/kg group were considered to be test substance-related. In the 900 mg/kg group, clonic convulsions (head and/or body twitches) were noted for a single male. No other test substance-related effects on open field parameters were noted for males treated with Imiprothrin. The mean time to first step (1.2 seconds) in the 1200 mg/kg Imiprothrin group was increased compared to the control group (0.5 seconds); however, this increase was attributed to the aforementioned male in the 1200 mg/kg group with a mean latency to first step of 6.3 seconds.

Permethrin: Test substance-related findings were noted during the open field observations for males that received Permethrin. Slightly impaired mobility and gait were noted for 3 males in the Permethrin-treated group. In addition to the mobility and gait impairment, these males were also noted with ataxia and/or slight to moderately coarse tremors. An additional male in the Permethrin-treated group was also noted with slight tremors. Significantly ($p<0.01$) increased mean time to first step and grooming counts were noted in the 200 mg/kg Permethrin group. Additionally, mean rearing counts in this group were significantly ($p<0.01$) lower than the control group. No other test substance-related effects on open field parameters were observed for males in the Permethrin-treated group.

Prallethrin: Slightly to moderately impaired mobility, clonic convulsions (consisting of back muscle twitching), slight to moderately coarse tremors, ataxia, and/or slightly to considerably impaired gait were noted for 2 and 1 males in the 250 and 400 mg/kg Prallethrin groups, respectively. No test substance-related effects on open field parameters were noted for males in the 150 mg/kg Prallethrin group.

Cyphenothrin: Five males in the 160 mg/kg Cyphenothrin group were noted with slightly impaired mobility and slightly to considerably impaired gait (gait score) during the open field observations; the difference from the control group was significant ($p<0.05$) for slightly

impaired mobility. As a result, significantly ($p<0.05$) fewer males in this group were noted with normal mobility and gait. In addition, 8 of 10 males in the 160 mg/kg Cyphenothrin group had slight or moderately coarse tremors, resulting in a significantly ($p<0.05$) lower number of males in this group with no observed tremors. One of the males in the 160 mg/kg group (noted with these findings) was euthanized in extremis following the FOB evaluation. Splayed or dragging hind limbs (inability to support weight) and/or clonic convulsions (consisting of back twitches), in addition to impaired gait, slightly impaired mobility, and moderately coarse tremors, were noted for 1 and 2 males in the 100 and 160 mg/kg Cyphenothrin groups, respectively; 1 of the 160 mg/kg group males was subsequently found dead. A dose-related decrease in mean rearing counts was noted in the Cyphenothrin-treated groups and an increase in mean urine pools was noted in the 100 and 160 mg/kg groups; differences from the control group achieved significance ($p<0.05$) in the 160 mg/kg group for urination. No test substance-related effects on open field parameters were observed for males in the 60 mg/kg Cyphenothrin group.

Deltamethrin: Six males in the Deltamethrin-treated group were noted with slightly or moderately impaired mobility, considerable or marked gait impairment (based on gait score), splayed or dragging hind limbs (inability to support weight), hunched body, ataxia, exaggerated hind limb flexion, and/or low arousal; differences from the control group were significant ($p<0.05$) for moderately impaired mobility and ataxia. As a result, significantly ($p<0.05$) fewer males in the Deltamethrin-treated group were noted with normal mobility and gait. In addition, a lower (not statistically significant) mean rearing count was noted in the 25 mg/kg Deltamethrin group compared to the control group. No other test substance-related effects on open field parameters were observed for males in the Deltamethrin-treated group.

Pynamin Forte/d-allethrin: The most prevalent test substance-related open field findings noted at the time of peak effect (2 hours following dosing) in the 200, 320, and 500 mg/kg Pynamin Forte groups included slightly impaired mobility, clonic convulsions (back-muscle twitching and/or head/body twitching [myoclonus]), slight to moderate tremors, and slight gait impairment (based on gait score). In the 500 mg/kg Pynamin Forte group, the aforementioned findings (with the exception of back-muscle twitching) were significantly ($p<0.05$) increased compared to the control group. As a result, corresponding reductions in the number of males with normal mobility and gait and absence of convulsions and tremors were noted in this group; differences from the control group were significant ($p<0.05$). In addition, dose-related increases in the mean time to first step were noted in the 320 and 500 mg/kg groups; differences from the control group were significant ($p<0.05$ or $p<0.01$). Furthermore, decreased mean rearing counts were noted in the 320 and 500 mg/kg Pynamin Forte groups; differences from the control group achieved significance ($p<0.05$) in the 320 mg/kg group only. No other test substance-related effects on open field parameters were observed for males in the Pynamin Forte-treated groups. An increase in mean urine pools was noted in the 320 mg/kg group; the difference from the control group was significant ($p<0.05$). However, because this finding did not occur in a dose-related manner, it was not considered to be test substance-related.

TABLE 7 . Summary Findings of Open Field Observations [no. animals showing sign and count data (mean±SD) from open field]

Treatment group	Time to first step (sec.)	Rearing	Urine pools	Grooming	Mobility	Convulsion	Tremor
Number Tested	10	10	10	10	10	10	10
Vehicle	05±0.07	3.6±1.96	0.4±0.70	0.4±0.84	0	0	0
Metofluthrin, 57 mg/kg	0.7±0.48	1.0±1.41†	0.6±0.84	0.2±0.42	1	0	2
Metofluthrin , 75 mg/kg	1.8±2.38	1.4±2.37†	0.4±0.70	0.9±1.29	3	0	5*
Imiprothrin, 900 mg/kg	0.5±0.15	3.6±2.37	0.8±0.63	0.7±1.34	0	1	0
Imiprothrin, 1200 mg/kg	1.2±1.81	2.9±3.0	0.3±0.48	0.3±0.67	1	0	1
Imiprothrin, 1500 mg/kg	0.7±0.26	3.6±2.59	0.2±0.42	0.3±0.67	0	0	0
Permethrin, 200 mg/kg	1.0±0.33*	1.0±1.63*	0.9±0.88	2.8±2.04*	3	0	4
Vehicle	0.6±0.14	3.2±2.9	0.4±0.52	0.4±0.7	0	0	0
Prallethrin, 150 mg/kg	0.4±0.08	1.7±2.41	0.6±0.84	0.5±0.97	0	0	0
Prallethrin, 250 mg/kg	0.7±0.31	2.2±2.30	0.8±1.03	0.4±0.97	2	0	2
Prallethrin, 400 mg/kg	1.1±1.73	2.7±2.98	1.1±0.74	0.1±0.32	1	1	1
Cyphenothrin, 60 mg/kg	0.5±0.09	2.3±3.16	0.4±0.52	1.7±1.95	0	0	0
Cyphenothrin, 100 mg/kg	0.7±0.40	1.5±1.84	0.8±0.42	0.8±1.14	1	1	1
Cyphenothrin, 160 mg/kg	0.7±0.14	0.4±0.52	1.1±0.74‡	0.7±1.06	5	1	8
Deltamethrin, 25 mg/kg	0.8±0.42	1.4±1.9	0.2±0.42	0±0.0	6	0	0
Vehicle	0.5±0.07	4.1±3.54	0.3±0.48	0.2±0.42	0	0	0
Pynamin Forte, 200 mg/kg	0.5±0.11	3.7±2.79	0.3±0.67	0.2±0.63	0	2	2
Pynamin Forte, 320 mg/kg	0.6±0.11†	0.8±1.14‡	1.1±0.99‡	0.4±0.7	1	4	4
Pynamin Forte, 500 mg/kg	0.7±0.16‡	1.9±2.42	0.4±0.53	0.3±1.0	4†	7†	8†

*Significantly different from control group at 0.01 using two-sample t-test
†Significanlty different from control group at 0.05 using Fisher's Exact test
‡Significantly different from control group at 0.05 using Dunnett's test
Data were obtained from Tables S13, S14 and S15 on page 104-110 of the study report.

4. Sensory Observations (see Table 8):

Metofluthrin: Test substance-related effects on sensory parameters were noted for the 2 males found dead in the 75 mg/kg Metofluthrin group. No reaction to tail pinch, touch, olfactory, and/or pupil stimulus, no forelimb/hind limb extension, more energetic response to the startle stimulus, and/or slightly uncoordinated air righting reflex were noted for these males during the sensory observations. In addition, 1 and 2 males in the 57 and 75 mg/kg Metofluthrin groups, respectively, had more energetic responses to the startle stimulus. No other test substance-related effects on sensory parameters were observed for surviving males in the Metofluthrin-treated groups. A significantly ($p<0.05$) fewer number of males in the 75 mg/kg Metofluthrin group were noted with a more energetic response to the tail pinch; however, the significance was attributed to an atypically high number of control group males with this finding and was not considered to be neurotoxicologically relevant.

Imiprothrin: Sensory observations were unaffected by administration of 900, 1200, and 1500 mg/kg Imiprothrin; no remarkable findings were noted at approximately 2 hours following dose administration. Significantly ($p<0.05$) fewer males in the 1200 and 1500 mg/kg Imiprothrin groups were noted with a more energetic response to the tail pinch and a higher number of males in these groups were noted to slowly turn and walk away following a tail pinch. However, the significance was attributed to an atypically high number of control group males with an energetic response and an atypically low number of control group males that slowly turned and walked away following a tail pinch; these findings were not considered to be neurotoxicologically relevant. In addition, 1 male in the 1200 mg/kg Imiprothrin group had a more energetic response to the startle stimulus; however, in the absence of a dose-related response, this finding was not considered to be test substance-related.

Permethrin: Two males in the 200 mg/kg Permethrin group either exhibited no response during the touch or pupil response observations and 1 of these males was noted with a slightly uncoordinated air righting reflex. No other test substance-related effects on sensory parameters were observed for males in the Permethrin-treated group. Significantly ($p<0.05$) fewer males in this group were noted with a more energetic response to the tail pinch and a higher number of males in these groups were noted to slowly turn and walk away following a tail pinch. However, the significance was attributed to an atypically high number of control group males with an energetic response and an atypically low number of control group males that slowly turned and walked away following a tail pinch; these findings were not considered to be neurotoxicologically relevant.

Prallethrin: Sensory observations were unaffected by administration of 150, 250, and 400 mg/kg Prallethrin; no remarkable findings were noted at approximately 2 hours following dose administration.

Cyphenothrin: The startle stimulus elicited a more energetic response for 3 males in the 160 mg/kg Cyphenothrin group than was observed for concurrent control group males. In addition, 1 of these males had no hind limb extension during the sensory observations. No other test substance-related effects on sensory parameters were observed for males in the Cyphenothrin-treated groups. One male in the 60 mg/kg group exhibited no response following pupillary stimulation; however, based on the lack of a similar effect at higher dose levels, this finding was not considered test substance-related.

Deltamethrin: Five males in the 25 mg/kg Deltamethrin group landed on their sides when the air-righting reflex was evaluated approximately 4 hours following dose administration; the difference from the control group was significant ($p<0.05$). As a result, the significantly

($p<0.05$) fewer males in this group had normal responses when air-righting reflex was evaluated. In addition, 2 of the aforementioned males had no hind limb extension during the sensory observations. Two males in the 25 mg/kg Deltamethrin group exhibited no response following approach stimulation; 1 of these males was also noted with a more energetic response to the startle stimulus.
No other test substance-related effects on sensory parameters were observed for males in the Deltamethrin-treated group.

Pynamin Forte/d-allethrin: No test substance-related effects on sensory parameters were observed for males in the Pynamin Forte-treated groups. All males in the 500 mg/kg group slowly turned and walked away following a tail pinch, resulting in 0 males with a more energetic response. When compared to the control group, the difference was significant ($p<0.05$) but not considered to be neurotoxicologically relevant. In addition, 2 males in the 320 mg/kg Pynamin Forte group had no response to pupillary stimulation; however, in the absence of similar effects at higher dose levels, this finding was not test substance-related. One male in the 500 mg/kg group had a more energetic response to startle stimulus; however, based on the lack of effects noted in this group during the sensory observations and because this finding was only noted in a single male, the more energetic response to startle stimulus was not considered to be related to test substance administration.

TABLE 8. Summary Findings of Sensory Observations (no. animals showing sign)

Treatment group	Touch response	Startle response	Tail pinch response	Pupil response	Hindlimb extension	Air righting reflex	Forelimb response
Number Tested	10	10	10	10	10	10	10
Metofluthrin, 57 mg/kg	0	1	0	0	0	0	0
Metofluthrin , 75 mg/kg	2	2	2	1	2	1	1
Imiprothrin, 900 mg/kg	0	0	0	0	0	0	0
Imiprothrin, 1200 mg/kg	0	1	2†	1	0	0	0
Imiprothrin, 1500 mg/kg	0	0	1†	0	0	0	0
Permethrin, 200 mg/kg	1	0	2†	1	0	1	0
Prallethrin, 150 mg/kg	0	0	0	0	0	0	0
Prallethrin, 250 mg/kg	0	0	0	0	0	0	0
Prallethrin, 400 mg/kg	0	0	0	0	0	0	0
Cyphenothrin, 60 mg/kg	0	0	0	1	0	0	0
Cyphenothrin, 100 mg/kg	0	0	0	0	0	0	0
Cyphenothrin, 160 mg/kg	0	3	0	0	1	0	0
Deltamethrin, 25 mg/kg	0	1	2	0	2	5†	0
Pynamin Forte, 200 mg/kg	0	0	3	0	0	0	0
Pynamin Forte, 320 mg/kg	0	0	1	2	0	0	0
Pynamin Forte, 500 mg/kg	0	1	0†	0	0	0	0

†Significanlty different from control group at 0.05 using Fisher's Exact test
Data were obtained from Tables S16, S17 and S18 on page 110-115 of the study report.

5. Neuromuscular Observations (see Table 9):

Metofluthrin: One male each in the 57 and 75 mg/kg Metofluthrin groups were noted with reduced hind limb resistance (indicative of weakness) or an absence of hind limb resistance, respectively. In addition, mean rotarod performance in the 75 mg/kg Metofluthrin group was 44.9% lower compared to the control group. No other test substance-related neuromuscular effects were noted in the 57 and 75 mg/kg Metofluthrin groups. One male (no. 72321) in the 75 mg/kg Metofluthrin group was found dead during the FOB assessments on study day 1.

Imiprothrin: Mean hind limb grip strength for the 1500 mg/kg Imiprothrin group was 37.5% lower than the control group at the time of peak effect (2 hours following dosing); the difference was significant ($p<0.01$). All other neuromuscular parameters were similar to the concurrent control group values.

Permethrin: Mean forelimb grip strength in the 200 mg/kg Permethrin group was 27.2% lower than the concurrent control group value; the difference was significant ($p<0.05$). All other neuromuscular parameters were similar to the concurrent control group values.

Prallethrin: Neuromuscular observations were unaffected by administration of 150, 250, and 400 mg/kg Prallethrin; no remarkable findings were noted at approximately 2 hours following dose administration. Mean rotarod performance in the 250 mg/kg group was slightly lower than the control group; however, in the absence of a dose response, the lower mean rotarod performance noted in this group was not test substance-related.

Cyphenothrin: Mean rotarod performance was 51.6% lower in the 160 mg/kg Cyphenothrin group compared to the control group; the difference was not statistically significant, but was considered to be test substance-related. All other neuromuscular parameters were similar to the concurrent control group values. No test substance-related neuromuscular effects were noted in the 60 and 100 mg/kg Cyphenothrin groups.

Deltamethrin: Mean forelimb and hind limb grip strength in the 25 mg/kg Deltamethrin group were 27.8% and 22.7% lower, respectively, compared to the control group approximately 4 hours following dose administration; differences were not statistically significant. However, the decreases were driven by animals in this group with impaired mobility and gait, and therefore considered to be test substance-related. No other neuromuscular effects were noted in the Deltamethrin-treated groups.

Pynamin Forte/d-allethrin: One, 1, and 2 males in the 200, 320, and 500 mg/kg Pynamin Forte groups, respectively, had reduced hind limb resistance, indicative of weakness. In addition, decreased forelimb and hind limb grip strength and was also noted in these groups compared to the concurrent control group. No other neuromuscular effects were noted in the Pynamin Forte-treated groups.

TABLE 9. Summary Findings of Neuromuscular Observations				
Treatment group	**Grip strength-forelimb (g)**	**Grip strength-hind limb (g)**	**Rotarod performance (sec)**	**Hindlimb footsplay (mm)**
Number Tested	10	10	10	10
Vehicle	518.2±164.45	226.1±88.46	103.2±35.87	54.4±10.60
Metofluthrin, 57 mg/kg	470.6±152.66	203.0±77.35	77.8±46.72	47.6±19.67
Metofluthrin , 75 mg/kg	435.6±144.75	166.9±49.29	56.9±60.11	45.4±18.73
Imiprothrin, 900 mg/kg	463.4±96.21	182.1±42.51	68.7±55.26	48.9±16.75
Imiprothrin, 1200 mg/kg	484.7±138.27	162.7±60.25	61.6±51.7	46.1±15.7
Imiprothrin, 1500 mg/kg	514.5±115.88	141.3±36.20‡	67.6±56.21	54.3±12.69
Permethrin, 200 mg/kg	377.1±81.37*	171.2±51.45	66.9±47.25	53.2±13.30
Vehicle	461.6±96.11	164.6±43.52	88.2±47.13	50.5±17.82
Prallethrin, 150 mg/kg	453.6±117.6	165.3±39.79	85.7±47.50	52.1±14.62
Prallethrin, 250 mg/kg	542.2±87.23	203.1±41.25	62.8±50.90	52.1±17.01
Prallethrin, 400 mg/kg	509.6±152.36	209.1±71.71	90.4±47.81	52.4±13.44
Cyphenothrin, 60 mg/kg	497.6±130.7	159.2±43.46	65.7±47.10	54.7±15.29
Cyphenothrin, 100 mg/kg	485.2±134.1	176.4±44.90	78.3±53.81	58.8±15.08
Cyphenothrin, 160 mg/kg	399.9±81.94	153.1±44.71	42.7±54.09	54.7±17.41
Deltamethrin, 25 mg/kg	333.1±224.57	127.2±59.64	60.8±62.41	40.5±15.00
Vehicle	459.8±135.15	165.6±38.82	88.1±47.85	45.6±16.06
Pynamin Forte, 200 mg/kg	377.2±58.32	125.8±41.64	86.8±48.27	37.4±14.47
Pynamin Forte, 320 mg/kg	348.6±145.68	153.2±58.93	73.9±48.73	43.0±18.02
Pynamin Forte, 500 mg/kg	308.2±129.25	129.9±34.77	81.3±48.59	44.8±19.70

*Significantly different from control group at 0.01 using two-sample t-test
‡Significantly different from control group at 0.05 using Dunnett's test
Data were obtained from Tables S19, S20 and S21 on page 116-118 of the study report.

6. Physiological Observations (see Table 10):

Metofluthrin: A test substance-related increased mean catalepsy time (3.7 seconds) was noted for males in the 75 mg/kg Metofluthrin group compared to the control group (0.6 seconds). This difference was primarily attributed to 1 male (no. 72348) in the 75 mg/kg group with a catalepsy time of 24.4 seconds. No physiological effects were noted in the 57 mg/kg Metofluthrin group.

Imiprothrin: An increased mean catalepsy time (4.4 seconds) was noted for males in the 1500 mg/kg Imiprothrin group compared to the control group (0.6 seconds). This difference from the control group was primarily attributed to 1 male (no. 72305) in this group with a catalepsy time of 37.6 seconds. However, in the absence of effects noted during the remaining FOB assessments, the increased catalepsy time noted for the single male (and subsequently the entire 1500 mg/kg Imiprothrin group), was not considered to be test substance-related. No physiological effects were noted in the 900 and 1200 mg/kg Imiprothrin groups.

Permethrin: A slight (0.8°C) increase (significant at $p<0.01$) in mean body temperature was noted for males in the 200 mg/kg Permethrin group approximately 4 hours following dose

administration. No other physiological effects were noted in the 200 mg/kg Permethrin group.

Prallethrin: No test substance-related physiological effects were noted in the 150, 250, and 400 mg/kg Prallethrin groups. A significantly ($p<0.01$) lower (0.9°C) mean body temperature was noted in the 400 mg/kg Prallethrin group compared to the control group at the time of peak effect. However, the difference was slight and the mean body temperature was comparable to other control group values noted in this study. All other physiological parameters were similar to the concurrent control group values.

Cyphenothrin: Dose-related increases (not statistically significant) in mean catalepsy time (1.2 and 1.5 seconds) were noted for males in the 100 and 160 mg/kg Cyphenothrin groups, respectively, compared to the control group (0.8 seconds) approximately 2 hours following dose administration. All other physiological parameters were similar to the concurrent control group values. No test substance-related physiological effects were noted in the 60 mg/kg Cyphenothrin groups.

Deltamethrin: Mean catalepsy time (2.1 seconds) in the 25 mg/kg Deltamethrin group was increased compared to the control group value (0.8 seconds); the difference was not statistically significant, but was considered to be test substance-related. In addition, a significantly ($p<0.05$) lower mean body temperature was noted in this group compared to the control group. Mean body weight in the 25 mg/kg Deltamethrin group was unaffected by test substance administration.

Pynamin Forte/d-allethrin: Increases (not statistically significant) in mean catalepsy time were noted for males in the 200, 320, and 500 mg/kg Pynamin Forte groups compared to the control group approximately 2 hours following dose administration. The males in the 320 and 500 mg/kg Pynamin Forte groups with increased mean catalepsy times were also noted with flattened posture, splayed hind limbs, and/or reduced hind limb resistance. These findings are indicative of weakness and reduced mobility; therefore, the increased mean catalepsy times noted in the 320 and 500 mg/kg Pynamin Forte groups were considered to be test substance-related. In the absence of other findings, the increased mean catalepsy time noted in the 200 mg/kg group was not considered to be test substance-related. All other physiological parameters were similar to the concurrent control group values. No test substance-related physiological effects were noted in the 200 mg/kg Pynamin Forte group.

TABLE 10. Summary Findings of Physiological Observations			
Treatment group	**Catalepsy (sec)**	**Body temperature (°C)**	**Body weight (g)**
Number Tested	10	10	10
Vehicle	0.6±0.25	37.7±0.55	177.1±20.69
Metofluthrin, 57 mg/kg	1.0±1.51	37.9±0.46	174.8±18.65
Metofluthrin , 75 mg/kg	3.7±7.83	38.1±0.57	178.2±19.66
Imiprothrin, 900 mg/kg	0.6±0.16	37.6±0.59	175.0±22.67
Imiprothrin, 1200 mg/kg	0.7±0.26	37.2±0.81	172.6±20.11
Imiprothrin, 1500 mg/kg	4.4±11.67	37.5±0.50	176.2±21.37
Permethrin, 200 mg/kg	0.6±0.21	38.5±0.55*	175.2±22.37
Vehicle	0.8±0.47	38.1±0.67	192.2±21.66
Prallethrin, 150 mg/kg	0.6±0.19	38.4±0.63	192.5±16.31
Prallethrin, 250 mg/kg	0.8±0.47	37.8±0.48	193.9±16.97
Prallethrin, 400 mg/kg	0.9±0.37	37.2±0.61‡	194.9±20.46
Cyphenothrin, 60 mg/kg	0.6±0.15	38.1±0.75	194.1±19.74
Cyphenothrin, 100 mg/kg	1.2±1.68	38.3±0.58	193.1±21.16
Cyphenothrin, 160 mg/kg	1.5±1.89	38.4±0.5	191.0±16.88
Deltamethrin, 25 mg/kg	2.1±2.86	36.7±1.83*	186.7±19.56
Vehicle	0.4±0.16	37.2±1.50	187.5±12.85
Pynamin Forte, 200 mg/kg	0.9±0.64	37.4±1.53	187.0±18.31
Pynamin Forte, 320 mg/kg	1.0±1.59	37.7±1.01	187.0±18.23
Pynamin Forte, 500 mg/kg	1.5±1.12	37.5±1.86	184.5±16.09

*Significantly different from control group at 0.01 using two-sample t-test
‡Significantly different from control group at 0.05 using Dunnett's test
Data were obtained from Tables S22, S23 and S24 on page 119-121 of the study report.

D. MACROSCOPIC EXAMINATIOS (Unscheduled Death):

The only internal macroscopic finding noted for animals that were found dead or euthanized *in extremis* were lungs not fully collapsed for male no. 72769 in the 320 mg/kg Pynamin Forte group. All other animals that were found dead or euthanized *in extremis* were internally normal.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATOR'S CONCLUSIONS:

The study author concluded that mortality and/or moribundity were noted at 75 mg/kg Metofluthrin, 160 mg/kg Cyphenothrin, and 320 and 500 mg/kg Pynamin Forte/d-allethrin. The predominant findings noted during the FOBs in the test substance-treated groups included tremors, clonic convulsions, impaired mobility, altered gait (body dragging, splayed or dragging hind limbs, hunched posture, and ataxia), and posture abnormalities, which were consistent with known effects of pyrethroids. In addition to the aforementioned CNS and neuromuscular effects, groups treated with Metofluthrin, Imiprothrin, Permethrin, Prallethrin, Cyphenothrin, Deltamethrin and Pynamin Forte elicited test substance–related effects on functional domains. These included physiological (red deposits on or around the nose and mouth and/or differences in mean body temperature), sensorimotor (consisting of no response to approach, touch, tail pinch, and pupil stimuli, a more energetic response to startle stimulus, and/or a lack of olfactory orientation), and autonomic effects (evidence of decreased grooming, lacrimation, and/or chromodacryorrhea).

B. REVIEWER COMMENTS:

The FOB results of this acute neurotoxicity study of seven pyrethroid compounds are consistent with those expected for Type I and Type II pyrethroids. These results show a continuation of the pyrethroids which were not among the 12 previously evaluated by the PWG. This study was conducted under the same conditions used in the PWG FOB study so that the classification based on clinical signs would be consistent.

Results summarized in Table 7 indicate that statistically significant effects on rearing in Metofluthrin treated male rats were observed at both doses used in this study. Other test substance-related effects were also observed in both dose groups. Thus, a dose which produced a no effect dose level was not achieved for Metofluthrin and lower doses should be used. For Imiprothrin, decreased hind limb grip strength was statistically significant only in the highest (1500 mg/kg) dose group (Table 9), however moderate to severe tremors were observed in all dose groups. Again, lower doses should be selected.

Appropriate dose ranges were used for the three other compounds tested. Prallethrin was tested using three doses (150, 250 and 400 mg/kg) in this study. These were minimally toxic doses. No statistically significant effects of Prallethrin were observed at any of the three doses used in this study (Tables 7, 8 and 9). There were also no test substance related changes in altered sensory observations, neuromuscular parameters or physiological effects at any of the three dose levels. Clonic convulsions, slight to moderately coarse tremors, ataxia, and slightly to moderately impaired mobility were observed in the mid-level (250 mg/kg) and high (400 mg/kg) doses, however. Only incidental effects were reported for the lowest (150 mg/kg) dose group, suggesting that a suitable dose range was chosen for Prallethrin. Cyphenothrin was tested at three doses in this study. A statistically significant increase in urine pools was observed only in the high (160 mg/kg) dose group, however dose related decreases in mean rearing counts were noted in both the 100 and 160 mg/kg dose groups. Test substance related effects were not observed in the lowest (60 mg/kg) dose group. Pynamin Forte was tested at three doses (200, 320 and 500 mg/kg) in this study. Significant increases in time to first step and decreases in mean rearing counts were observed in both the 320 and 500 mg/kg dose groups. No test substance-related effects were reported for the lowest (200 mg/kg) dose group. Tetramethrin and d-phenothrin (sumithrin) were not

included in the main FOB study since in the range-finding study neither showed any clinical signs at 5000 mg/kg (or higher doses) the maximum dose used in each experimental condition. The observed potency of the tested pyrethroids is as follows: deltamethrin > metofluthrin> cyphenothrin> permethrin> prallethrin>d-allethrin>imiprothrin

TABLE 11. FOB Pyrethroid

Test article	Structural element	FOB Classification
Vehicle	Corn oil	-
Metofluthrin	Non-cyano	Type I
Imiprothrin	Non-cyano	Type I
Permethrin [a]	Non-cyano	Type I
Prallethrin	Non-cyano	Type I
Cyphenothrin	Cyano	Mixed
Deltamethrin [a]	Cyano	Type II
Pynamin Forte (d-allethrin)	Non-cyano	Type I

a= Permethrin and Deltamethrin represented Type I and Type II positive control groups, respectively.

C. PROTOCOL DEVIATIONS

Two amendments were made to the study protocol (Appendix A, Pages 126-131). Amendment 1 modified the protocol by 1) addition of a report date for the Audited Report Date and 2) to clarify the storage conditions for the formulations used in the study. Amendment 2 modified the protocol by clarifying the statistical methods used for the positive control compounds which were compared to the control group using the two-sample t-test. None of these modifications significantly affected the outcome of the study.

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